

PCR Protocols Methods and Applications

Edited by
David H. Gelfand, John J. Sninsky, and
Thomas J. White

The polymerase chain reaction (PCR) is a powerful new method with widespread applications in diagnosis. With over fifty chapters of this unique, comprehensive benchtop reference, complete range of PCR methods and Equipment, reagents, and supplies are

Key features include:

- Comprehensive survey of PCR protocols, from multiplex PCR methodology
- Procedures, with details on reagents and equipment
- Laboratory set-up
- Preventing contamination

Illustration by E. Calver, courtesy of Cetus Corporation.

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A Guide to Methods and Applications

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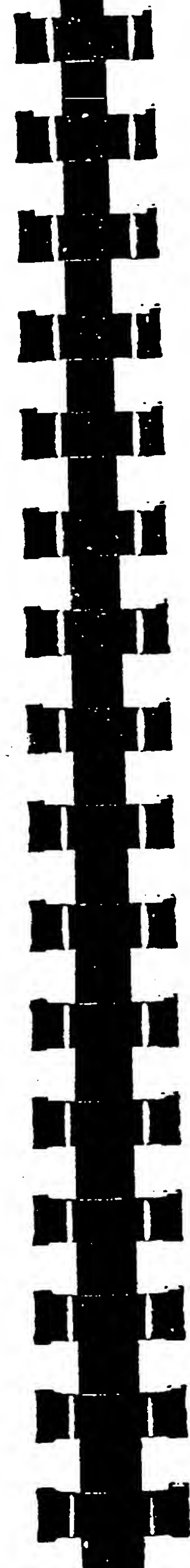
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sample handling, from sample collection to sample extraction. The following are additional precautions that should be taken:

1. Change gloves frequently.
2. Quick spin tubes before opening them.
3. Uncap and close tubes carefully to prevent aerosols.
4. Minimize sample handling.
5. Add nonsample components (mineral oil, dNTPs, primers, buffer, and enzyme) to the amplification reactions before the addition of sample DNA. Cap each tube after the addition of DNA before proceeding to the next sample.

Judicious Selection of Controls

First, for use as a positive control, select a sample that amplifies weakly but consistently. The use of strong positives will result in the unnecessary generation of a large amount of amplified sequences. If plasmid DNA containing the target sequence is used as a positive control, it should be substantially diluted. Depending on the detection system used, as few as 100 copies of target will suffice as a positive control. Second, use well-characterized negative controls. The extreme sensitivity of PCR may enable the detection of nucleic acid sequence from a sample that is negative by all other criteria. Third, include multiple reagent controls with each amplification. Because the presence of a small number of molecules of PCR product in the reagents may lead to sporadic positive results, it is important to perform multiple reagent controls. The reagent controls should contain all the necessary components for PCR but without the addition of template DNA. This system has proved to be extremely sensitive in detecting the presence of contaminants, as the absence of exogenous DNA enables the efficient amplification of just a few molecules of contaminating sequence.

Although amplified products are most problematic, other potential sources of contamination/carry-over need to be considered, especially when additional manipulations of the amplified DNA are performed. The cloning of amplified product is a case in point. Often, the amount of target generated from an amplification is insufficient for direct cloning and requires re-amplification of the target. To minimize re-amplification of nonspecific products, the band of interest

is first separated on a gel, excised and a subsequent amplification. potentially result in cross-contamination. For example, gel contamination. For example, gel in 1 N HCl to deplete any residual device should be used to cause the surfaces of UV transilluminated, a sheet of plastic wrap should the gel from the surface of the controls that have been amplified with the sample of interest should no preparative-gel.

The list below highlights other sources of contamination:

1. Plasmid or phage DNA containing
2. Purified restriction fragment of
3. Dot blot apparatus
4. Microtome blades
5. Centrifuges
6. Speed Vacs/vacuum bottles
7. Dry ice/ethanol baths

Other sources of contamination: during (the preparation of sample: similar care) will most certainly here will serve as a guide in implementation if not eradicate carry-over

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